Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides†

Ralph C Kirkwood

Graduate School of Environmental Studies, University of Strathclyde, The Wolfson Centre, Taylor Street, Glasgow, G4 0NW, Scotland, UK

Abstract: The plant cuticle is a highly complex membrane which forms the outer surface of the aerial portion of plants. The nature of the plant cuticle is reviewed with particular regard to its action as a potential barrier to the penetration of pesticide molecules; the role of the cuticular waxes is highlighted. The physicochemical properties of the cuticle influence the behaviour of spray droplets and, in turn, may affect the rate and efficiency of cuticle penetration. The permeation of active ingredients is influenced by their solubility characteristics as indicated by octanol/water (log K_{ow}) and cuticle/water (K_{cw}) partition coefficients. Penetration of hydrophilic compounds (low log K_{ow}) may be enhanced by hydration of the cuticle, while transcuticular transport of non-polar solutes (high log K_{ow}) is increased by factors which reduce wax viscosity. The use of in-vitro models involving isolated cuticle membranes, isolated cuticle waxes, or isolated leaves has helped to focus on the activities of the cuticle in the absence of other physiological factors. Using these systems, the role of the waxes as a transport-limiting barrier has been identified and the factors influencing sorption, permeance and desorption examined. The action of surfactants, *in vitro* and *in vivo*, has been briefly addressed in regard to their role in facilitating cuticle penetration; other steps involving surfactant/solute/cuticle are complex, and synergy appears to depend on a number of factors including test species, concentration of active ingredient, surfactant type and concentration. Adjuvants may greatly influence the surface properties of the droplet, predispose the cuticle to solute transport, and enhance pesticide activity. The nature of these complex inter-relationships is discussed.

Keywords: plant cuticle; physicochemical properties; herbicides; pesticides; penetration; partitioning; adjuvants; surfactants

1 INTRODUCTION

In the case of foliage-applied herbicides, delivery of active ingredients to the target site depends on the efficiency of a number of inter-related factors including the efficiency of cuticle retention and penetration, absorption into the leaf tissues and possibly translocation from the sites of absorption.[1] The importance of the cuticle as the first, if not the most important, barrier to penetration of xenobiotics is well documented.[2–5] Among a range of factors, the physicochemical characteristics of the epicuticular waxes appear to be of particular significance since they may affect the retention and distribution of the active ingredient (AI). In addition, the heterogeneous nature of the cuticular membrane may necessitate a series of partitioning steps in transcuticular transport of xenobiotics. In this paper the structure and function of the plant cuticle will be considered, with particular reference to the role of the waxes, in relation to the sorption and transport of solutes across the cuticle. Recent developments which contribute to our understanding of these processes and the roles of adjuvants will be reviewed.

2 THE PLANT CUTICLE

2.1 Cuticle structure

The plant cuticle is a thin continuous layer (<0.1–10 μm) of predominantly lipid material synthesised by the epidermal cells and deposited on their outer walls. The structure and ontogeny of plant cuticles has been recently reviewed by Holloway[6] and Jeffree.[7]

The major structural model is a bilayer cuticular membrane in which the two layers are distinguishable by their ontogeny, ultrastructure and chemical composition.[7] The outer region is composed mainly

* Correspondence to: Ralph C Kirkwood, Graduate School of Environmental Studies, The Wolfson Centre, Taylor Street, Glasgow, G4 0NW, Scotland, UK

† Based on a paper presented at the meeting 'Uptake of Agrochemicals and Pharmaceuticals', organised by AH Cobb, AM Stalker and DJ Livingstone on behalf of the Pesticides Group and the Physicochemical and Biophysical Panel of the SCI and the Strathclyde Institute for Drug Research and held on 9 December 1997 at 14/15 Belgrave Square, London.

(*Received 9 February 1998; revised version received 29 June 1998; accepted 17 August 1998*)

 Pestic Sci 0031-613X/99/$17.50

of soluble and polymerised aliphatic lipids, while the inner layer, which forms by impregnation of the cell wall, contains large amounts of various cell-wall polysaccharides. Adjacent to the cuticular membrane is a layer of pectin-rich cell-wall polysaccharides called the pectin lamella.

The principal component of the cuticular membrane is the lipid polyester cutin within which are found lamellae and fibrillae.[6] The latter are composed of polysacharides which may exhibit a distinctive reticulate pattern while the lamellae may contain wax compounds. Considerable inter-specific variation has been observed in cuticle fine structure, ranging from essentially amorphous to predominantly lamellate or reticulate. The major differences between cuticle structural types have been ascribed by Jeffree[7] to the relative extent of the development of the cuticular membrane and of the underlying cell wall rather than to fundamental differences in mechanism or biochemistry.

The fine structure of isolated leaf cuticles of ivy (*Hedera helix* L) has been studied using atomic force microscopy.[8] The lamella and reticulate zones, distinguishable by transmission electron microscopy, were also observed with atomic force microscopy. The reticulate zone appeared to consist of fibrils of around 50 nm in diameter and these were shown to be polysaccharide in composition.

Cutin is an insoluble polyester of cross-linked hydroxy-fatty acid and hydroxyepoxy-fatty acids and waxes.[6] The precise intermolecular structure of the cutin polymer matrix is still uncertain, though two major lipid polymers appear to be present.[7] In addition to polyester cutin, 'cutan' is believed to be a polymethylene polymer which may be the principal polymer in the cuticles of certain species.

2.2 Cuticular waxes

Riederer and Markstädter[9] have critically reviewed current knowledge of cuticular waxes which are the main functional components of the primary aerial surfaces of higher plants. Removal of cuticular waxes from isolated plant cuticles leads to an increase in permeability of several orders of magnitude. Recent comprehensive reviews of their chemical composition and biosynthesis include those by Kolattukudy and Espelie,[10] Bianchi,[11] and Kolattukudy.[12]

Cuticular wax is extracted by dipping intact organs (leaves, fruits, stems) into an organic solvent or by extracting isolated cuticular membranes. Analyses of samples of such waxes will tend to give results biased in favour of the component(s) making up the epicuticular aggregates.[9] Additional problems may occur due to interspecific variations in extraction yields, in surface yields, and discrimination in favour of certain wax constituents; where possible cuticular membranes rather than whole leaves should be used as sources of wax samples.

While capillary gas chromatography and mass spectrometry have enabled major progress to be made in the chemical analysis of plant cuticular waxes, a number of problems have been described by Riederer and Markstädter.[9] For example, alkanals are especially difficult to analyse when compounds having hydroxy and carboxy groups are present in the sample. These problems can be overcome by separating samples of cuticular wax containing alkanals into non-polar and polar fractions by a solid-phase extraction step.

The amount and composition of plant cuticular waxes are subject to the influence of endogenous and exogenous factors.[9] Light, temperature and humidity may influence the coverage and composition of cuticular waxes and possibly the fine structure of epicuticular aggregates.[13,14] Leaf age has a prominent effect on wax amounts and composition.[15] Certain major constituents of plant cuticular waxes may undergo spontaneous chemical reactions while others are unaffected.[15] The alkanal coverage of leaves of *Fagus sylvatica* L increases as long as leaf expansion and wax biosynthesis proceed, but declines during the mature state of the leaf. Composition of leaf waxes should be determined as a function of age.

Epicuticular waxes, which cover the leaf surface, are formed in the epidermal cells[16] and pass through the epidermal cell membrane, cell wall and the cuticle to the surface. The method of wax deposition is controversial and two hypotheses have been proposed ('pore' v 'diffusion'). The pore hypothesis states that pores in the cuticle provide a path for wax to move to the plant surface. Pores have been identified in the leaves of several species by a number of investigators.[17–19] The diffusion hypothesis states that wax dissolved in a volatile solvent diffuses through the cell wall and cuticle and crystallises at the surface on contact with air.[20,21] Evidence to support this hypothesis has been reported.[22–24] Recently, Anton *et al*[24] found evidence that distribution of ^{14}C-labelled waxes occurred randomly throughout the cuticle of broccoli (*Brassica oleracea* L. var. capitata); there was no evidence to support the hypothesis of wax-depositing pores.

Wax synthesis involves condensation of 16- and 18-carbon fatty acids with malonyl-CoA by acetyl-coenzyme A fatty acid elongases in NADPH-dependent reactions. Application of the thiocarbamate herbicide triallate dramatically reduced ECW deposition in susceptible but not resistant wild oats (*Avena fatua* L).[25]

2.3 Epicuticular waxes and surface wetting

Epicuticular wax covers the leaf surface of many higher plants and its hydrophobic nature makes it difficult to obtain good wetting of leaf surfaces with aqueous sprays.[26,27] Deposition, distribution and retention of spray droplets are affected by the extent of coverage and chemical composition of the epicuticular wax and by leaf surface microroughness.[26] High levels of microroughness may result in air/liquid and solid/liquid interfaces which inhibit herbi-

cide penetration. Leaf surface microroughness is caused by epicuticular waxes, cell surface contours, leaf venation and trichomes. Trichomes may be of several types including prickles, microhairs and bicellular trichomes.[27] In johnsongrass (*Sorghum halepense* (L) Pers) bicellular trichomes initially increased leaf microroughness. With increasing leaf maturity, however, trichomes discharge mucilage that spreads over the epicuticular wax crystals, decreasing microroughness and presenting another barrier to herbicide penetration. Drought stress greatly increased epicuticular wax weights and formation.

Droplet retention is known to be a function of surface tension and the advancing and receding contact angles of a droplet; these are related to the chemical composition and microroughness of the leaf-surface. A retention model for droplets on solid surfaces has been described by Watanabe and Yamaguchi.[29] Such models can be useful in optimising differential retention and improving the formulation of pesticides. A spray deposition model based on the fundamental surface adhesion equation of Thomas Young (1805), has also been described and further evaluated, giving a good understanding of the factors involved.[30,31]

3 CUTICLE PENETRATION

3.1 Mechanisms

Transport through the cuticle is a diffusion-controlled process consisting of three components involving: (1) sorption into the cuticle, (2) diffusion through the cuticle, and (3) desorption from the cuticle.[32,33]

Sorption is the initial interaction of an applied pesticide with the plant cuticle which can be assessed directly by measuring the concentration in the cuticle after removal of the surface material. The partition coefficient (K) can be determined from

$$K_{\text{cuticle/solvent}} = \frac{\text{Concentration in the cuticle}}{\text{Concentration in the solvent}} \quad (1)$$

Bukovac and Petracek[33] found that sorption of naphthalene acetic acid (NAA) by tomato fruit cuticular membrane was linearly related to the equilibrium concentration of NAA in the sorbate solution. In contrast, sorption isotherms for the surfactant 'Triton' X-100 were multiphasic; the sharp initial increase in sorption was followed by a plateau typical of sorbents having a finite number of sorption sites. A subsequent increase was interpreted as new sites becoming available due to re-orientation of surfactant molecules or their formation of multilayers.[33]

Transcuticular transport studies have involved the use of infinite[33–36] or finite[37] dose systems. In the former the diffusion of a pesticide is monitored from an aqueous donor of essentially constant concentration, across the cuticle to an aqueous receiver. Typically, the amount of solute which diffuses across a cuticle of given area in given time is measured as a function of the concentration gradient (driving force) across the membrane. In the case of finite systems a given dose of pesticide is applied to an agar block or directly onto the isolated cuticle which is located on an agar block which acts as receiver. Such a system enables monitoring of cuticle penetration from droplets or deposits under controlled conditions; considerable variation has been observed with this system.[33]

Desorption is the final event in transfer across the cuticle. If sorption and desorption isotherms are not superimposable, the phenomenon is referred to as hysteresis, it may reflect covalent bonding, ionic bonding or the existence of van der Waals forces. Such non-reversible interactions may increase lag times and reduce penetration.[33]

3.2 Model systems

Model systems have been used to study cuticle penetration involving isolated cuticular membranes,[33,38] isolated conifer leaves[39] and recrystallised cuticular waxes.[40–42] Uptake of lipophilic organic compounds for aqueous solutions in conifer needles proceeded in two distinct phases.[32,39] The first, rapid, phase was attributed to sorption of the chemicals to the needle surfaces and the second, slower, phase, possibly reflected penetration across the cuticles and accumulation in the needle interior. Penetration rate was linearly proportional to the cuticle/water partition coefficients (K_{cw}) of the chemicals and the specific surface areas of the needles. The results of these studies suggested that foliar uptake involved four consecutive stages consisting of (1) leaf surface sorption, (2) diffusion into the surface waxes, (3) diffusion across the cuticular membrane and (4) diffusion across the cell walls (apoplast) and accumulation in the symplast.

The distribution of pentachlorophenol (PCP) and 2,4-dichlorophenoxyacetic acid (2,4-D) in conifer needles was studied following uptake from aqueous solutions for periods of < 21 h.[32] Desorption kinetics revealed two compartments (C_1 and C_2) in which organic compounds were sorbed reversibly and one compartment (C_3), possibly apoplast or symplast, from which organic solutes could not be desorbed. Reversible desorption was rapid, achieving equilibration in < 1 h and was attributed to sorption to the surfaces of cuticular waxes and sorption in waxes and cutin. Distribution between these compartments varied with time of loading and depended on plant species and the properties of the solutes.

In isolated plant cuticles, solutes which have been sorbed to the inner amorphous compartment can be desorbed via the outer transport-limiting barrier.[43] This enables calculation of rate constants of desorption which are independent of initial partitioning and

solute lipophilicity. Rate constants are strongly dependent on the molar volumes of the solutes, indicating the importance of solute mobility within the transport-limiting barrier of the cuticle.

The use of recrystallised leaf waxes to investigate the specific transport properties of cuticular waxes of barley leaves has been reported by Schreiber and Schönherr[40] and Schreiber.[41] A small amount of radiolabelled substance (0.01–0.1% by mass) was added to the wax before crystallisation and subsequent desorption was measured. The desorption kinetics were non-linear and asymptotically approached 100% desorption. Using ^{14}C-labelled octadecanoic acid as a molecular probe, the transport properties of cuticular waxes from a range of plant species were characterised.[42] The diffusion coefficients were lowest in the wax isolated from perennial leaves, intermediate in plants with deciduous leaves and highest in fruit waxes. Comparison of diffusion coefficients in cuticular waxes with those in biomembranes showed that the former were much lower (five to seven orders of magnitude). Schreiber and Riederer[42] concluded that (1) a model system using cuticular waxes reflected the transport properties of the intact cuticular membrane, (2) the barrier properties of intact cuticles were almost solely a function of cuticular waxes, (3) the waxy transport-limiting barrier of the cuticle functioned like a sorption/diffusion membrane, (4) this experimental system could be used to determine cuticular permeability in the case of stomatous cuticles or where cuticle isolation was not possible.

Spectroscopic investigations have revealed that the structure of wax contains two distinct phases (amorphous and crystalline).[44,45] The crystalline wax appears to consist of linear long-chain aliphatic molecules forming more or less perfect crystals, while the amorphous wax phase is believed to be composed of the chain ends of the aliphatic molecules. Diffusion across the transport-limiting barrier is thought to occur only in the amorphous wax phase and a molecular model for such diffusion has been proposed.[46] The amorphous phase provides a tortuous diffusion path across the wax, and the crystalline wax phase can be regarded as an excluded volume with respect to diffusion.[47] Schreiber *et al*[47] examined the diffusion coefficients of various molecules in the reconstituted cuticular waxes of beech (*Fagus sylvatica* L) and spruce (*Picea abies* (L) Karst). In most cases the diffusion coefficient was significantly lower in spruce than in beech and it was concluded that the barrier properties of spruce wax were enhanced by increased wax crystallinity, with a resulting higher tortuosity for permeating solutes. Schreiber *et al*[48] have reviewed diffusion of substances through cuticles with particular reference to the basic principles and equations.

Recent studies by Schönherr and Baur,[49] Baur *et al*[50] indicated that the diffusion path of solutes within the transport-limiting barrier of the cuticle has a large tortuosity which is reduced or removed when the waxes are removed.

4 THE EFFECT OF ADJUVANTS ON CUTICLE PENETRATION

4.1 In-vitro studies

The active ingredients (AI) of pesticides are sometimes formulated using a range of additives, known as adjuvants. These affect the physical properties of the spray liquid and may act as emulsifiers, wetting agents, spreaders, stickers, antifoaming agents or buffers.[51] In addition adjuvants such as surfactants may act as enhancers of cuticle penetration by predisposing the cuticular membrane to solute transfer or by acting as co-solvents.[52]

Schönherr[53] examined the effects of alcohols, glycols and monodisperse ethoxylated alcohols on the mobility of 2,4-D by unilateral desorption from the outer surface (UDOS) using isolated plant cuticles of mature bitter orange (*Citrus aurantium* L). The results showed that 1-heptanol, 1-octanol and 1-nonanol increased 2,4-D mobility by 25- to 30-fold. Increasing the number of C atoms in the alcohols decreased their effectiveness as did increasing ethoxylation; free glycols had no effect on 2,4-D mobility. These findings suggested that alcohols and ethoxylated alcohols are sorbed in cuticular waxes and plasticise them. The results also showed that alcohols and ethoxylated alcohols having between 6 and 10 carbon atoms are powerful accelerator adjuvants provided that the degree of ethoxylation is not too high; ethylene glycols had no effect on solute mobility in the cuticular membrane. With increasing molecular weight of alcohols and ethoxylated alcohols, effects on mobility decreased and lag times increased. The practical implications of this study have been outlined:[53]

- Solubility and diffusivity of AI in the cuticle waxes are usually rate-limiting in foliar uptake.
- The only approach to increase rates of uptake lies in manipulating the diffusivity of the AI in the cuticle once wetting, retention and partitioning (which contributes to the outer phase boundary resistance) have been optimised.
- The solubility and diffusivity of an AI in cuticular waxes is enhanced and can be greatly manipulated by nonionic alcohol ethoxylates.
- Penetration of AIs for which cuticles are particularly efficient barriers (large AIs) will be accelerated more than those penetrating rapidly.

Recently Schönherr and Baur[54] have reviewed the mechanisms involved in the transfer of solutes across cuticle membranes and the effect of surfactants and other adjuvants on the rates of uptake of orgnic compounds. Their earlier work involved the use of octanol/water (K_{ow}) and cuticle/water (K_{cw}) partition

coefficients in modelling the permeance of cuticles.[55–57] This approach has been questioned,[54] since wax/water partition coefficients (K_{wxw}) are much lower and probably account better for differential solute solubility in the limiting skin of the cuticle.[40,47] However, uptake of agricultural sprays occurs not from water, but from hydrated formulation residues.[49] Thus K_{wxfr} is the appropriate partitioning coefficient which characterises differential solubility in the waxy limiting skin and in the formulation residue, while the cuticle/water (K_{cw}), the polymer matrix/water (K_{mw}), or the octanol/water (K_{ow}) partition coefficients can account for differential solubility between the sorption compartment and water in the cell wall.[54] Taking account of these considerations, an equation has been derived which relates the rates of solute flow across the cuticular membrane to properties of solutes, adjuvants, limiting skins and cuticles.[54]

$$J = k^* \cdot l_{ls}(K_{wxfr} \cdot C_{fr} - K_{mxw} C_{apo}). \qquad (2)$$

where J = rate of penetration. The term in parenthesis represents the driving force, composed of differential solubilities in the wax and formulation residue (K_{wxfr}), polymer matrix and water (K_{mxw}), together with the concentrations in the formulation residue (C_{fr}) and in the apoplast (C_{apo}), respectively. In situations where uptake into cells and translocation are rapid and the solute concentration in the apoplast remains negligible, the term ($K_{mxw}C_{apo}$) disappears and the driving force depends only on the product $K_{wxfr}C_{fr}$. This approach separates the effects of adjuvants on solute mobility (k^*) from effects on differential solubility and driving forces ($K_{wxfr}C_{fr}$).[54] Schönherr and Baur[54] examined the effects of certain accelerator adjuvants on penetration of 2,4-D. Monodisperse ethoxylated decanols were examined at high surfactant concentrations (0.1 M) and $C_{10}E_2$ was found to be the most effective, with rates decreasing as ethoxylation increased. At low surfactant concentration (0.001 M) this order was reversed with $C_{10}E_8$ giving maximum rates. Tributyl-phosphate was also a powerful accelerator of 2,4-D penetration. They concluded that, providing maximum driving forces are realised, rates of penetration of an organic compound can be further increased by adding accelerator adjuvants. Accelerator performance depended on plant species, type of solute, the amount of accelerator and other adjuvants. The type and amount must be selected to ensure that accelerator and AI penetrate at similar rates. The optimum concentration of accelerator varied according to the amount of wax per unit area of leaf.

In their studies powerful accelerators included pentafluorophenol, alkyl esters of phosphoric acid, dicarboxylic acid esters and the insecticide chlorfenvinphos. The rates of penetration of accelerators depended on (1) the properties of the waxes of plant species, (2) molar volumes of accelerator, and (3) the intrinsic accelerating properties of the adjuvant.[54] Rates of diffusion of an AI in the waxes increased with increasing concentrations of accelerator sorbed into the waxes[50] and accelerator effects increased at lower temperatures.[54,55]

Accelerators work by temporarily decreasing the viscosity of cuticular waxes, thus enhancing solute mobility and reducing temperature effects. At low temperatures, energies of activation for diffusion in cuticles are very high leading to low permeabilities and rates of penetration;[49,56] the use of accelerators in low-temperature formulations can restore rates of penetration to levels nearly as great as at 20°C.[54]

Baur *et al*[57] examined the mechanistic aspects of temperature effects on cuticles and cuticular waxes. They measured the mobilities of lipophilic organic solutes in cuticular membranes isolated from mature leaves of a range of species over a temperature range of 15 to 78°C. Solute mobilities increased >1000-fold, corresponding to temperature coefficients (Q_{10}) of 3–14. No distinct phase transitions were observed due to sudden changes in the cuticular membrane, though rearrangement of at least some wax constituents was evident at high temperatures. Depending on species and solute size, activation energies of diffusion (E_D) ranged from 75 to 189 kJ mol^{-1}; variability between cuticles and effects of the molecular size of AIs decreased with increasing temperature. There was evidence that organic solutes differing in molecular size (130–349 cm^3 mol^{-1}) and K_{cw} (25–108) used similar diffusion paths in the cuticular membranes of all twelve plant species involved. This implies that diffusion occurs in regions with identical physicochemical properties and differs only in magnitude.[57]

Solute mobility varies according to plant species, but only two solute properties can account for differences between solutes which are not accelerators *per se*;[58] these are molar volume and structure (aliphatic or cyclic). Schönherr and Baur[59] have examined a range of aliphatic and cyclic compounds varying in molar volumes to determine the plasticising effects of monodisperse C_8E_4 in isolated leaves. Average concentrations of C_8E_4 in cuticles and cuticular waxes amounted to 8.6 and 0.86%, respectively and solute mobility was increased by less than 136-fold. This surfactant effect increased with molar volume of five solutes containing ring systems (2,4-D, NAA, triadimenol, tebuconazole, bitertanol); effects on aliphatic acids were lower compared to cyclic components of comparable molecular volumes; solute mobilities in cuticles caused by differences in solute size were greatly reduced by C_8E_4. Furthermore, since water permeability and organic solute mobility in plant cuticles show a log normal distribution, addition of accelerators modifies both phenomena such that normal distributions are observed, by reducing the numbers at the slow tail of the distribution.[60]

The use of seed oils as adjuvants to increase the efficacy of post-emergence herbicides is well documented.[61–64] Their effect on the penetration of [^{14}C]quizalofop-P-ethyl (log K_{ow} 4.5) and [^{14}C]fenoxaprop-P-ethyl (log K_{ow} 4.58) has been studied using isolated cuticles of three test species.[65] Seed oil enhanced the transfer of AI through cuticles and this may be related to the ability of the oils to partition into the cuticle, as has been previously observed for non-ionic surfactants.[66,67] Enhanced diffusion may result from increased fluidity of the cuticular components,[63] as suggested previously for nonylphenyl surfactants with a low ethylene oxide (EO) content.[68] Seed oil may solubilise epicuticular waxes[69] but this was not detected in the above study.

4.2 In-vivo studies

Isolated cuticles are a valuable tool with which to study the mechanisms involved in foliar penetration. They represent simpler systems than in-situ cuticles since they are not exposed to the physical and physiological influences of the living tissue.[39] There are a number of limitations to the use of isolated cuticles including:

(1) only thick, astomatous, cuticles can sustain the mechanical strains of isolation, thus excluding most species (crops and weed) of economic importance including most *Gramineae* species;
(2) in preparation, isolated cuticles are shaken for prolonged periods in liquid solutions and damage to the epicuticular waxes may greatly influence the behaviour and effect of adjuvants.

For these and other reasons, studies with isolated cuticles should, where possible, be complemented using intact plants; the latter can provide an overview of the sequence of progress which ultimately results in target-site delivery.

The effect of adjuvants on foliar uptake generally has been studied using radiolabelled organic chemicals and intact or detached leaves.[70,71] The approach may be limited to measuring rates of disappearance of solutes from the leaf surface[52,72] and has the disadvantage that the effects of adjuvants on cuticle permeability and uptake cannot be separated.

Many studies of surfactants have been carried out by Holloway and co-workers.[73–75] Stock *et al*[76] suggested that several sites of action may be involved when activation involves penetration of the surfactant. These may be located in the cuticle *per se*, the outer epidermal wall and the deeper internal tissues of the treated leaf. They suggested that the surfactant may interact with barriers which impede diffusion of the compound, removing sites of adsorption, and changing their properties. Thus, preconditioning sites or pathways may reduce resistance to penetration and facilitate absorption into the leaf. Localisation studies, *in situ*, indicate that only small amounts of low EO surfactants (relatively lipophilic) are likely to be taken up into the outer layer which incorporates the cuticle. Larger amounts of higher EO surfactants may be retained temporarily in the epidermis, increasing hydration and facilitating penetration of more hydrophilic compounds.[75]

Studies with a range of compounds of varying low K_{ow} and surfactants of varying EO contents provided data[73,74] which support the predictive response model originally proposed by Holloway and Stock[76] and modified by Stock *et al*.[72] Studies using [^{14}C]asulam (log K_{ow} 0.3), [^{14}C]diflufenican (log K_{ow} 4.6) and a range of nonylphenyl surfactants (EO 4–14) applied to fronds of bracken (*Pteridium aquilinum* (L) Kuhn) also supported the model.[77] Earlier confirmation of the importance of surfactant mean EO number was evident from isolated cuticle studies with [^{14}C]isoproturon and two nonylphenyl surfactants.[78]

There is evidence that non-ionic surfactants may penetrate the cuticle relatively rapidly and in large amounts,[79,80] causing effects on swelling and hydration of the cuticular membrane and on plasticising or solubilising of the waxes.[53,81] These effects may explain the enhanced penetration of polar and nonpolar pesticides, respectively. At concentrations above the critical micelle concentration (CMC), however, surfactants may retard the rate of cuticular penetration of lipophilic solutes.[40] This can occur when the solute is more soluble in the surfactant micelles than in water; substantial amounts of AI may partition into these micelles. This results in a reduced partition coefficient between the cuticle and the deposit ('K depression'). Activator surfactants must compensate for such detrimental effects by enhancing the mobility of AI in the transport-limiting barrier by changing the state or amount of soluble cuticular lipids.[40]

Baur *et al*[82] have pointed out that, for foliar uptake of pesticides, cuticle/water or octanol/water partition coefficients are useful predictors only during the early stages of application where water is present. The liquid phase in contact with the cuticle is not simply water, and partitioning occurs between adjuvant residue and the cuticle. These partition coefficients have to be known for comprehensive analysis and modelling of the uptake of pesticides. Using isolated cuticles from a range of leaves and fruits, they determined the partition coefficients of AI between plant cuticle and glycerol or poly(ethylene glycol) 400 (PEG 400) and the relationship of these with foliar uptake. K_{CGLY} and K_{CPEG} values of seven organic compounds (log K_{ow} 0.8–6.1) were obtained. Glycerol was a better solvent for lipophilic solutes than water, but PEG 400 was a good solvent for polar and nonpolar solutes.

5 CONCLUSIONS

Recent developments have helped our understanding of the plant cuticle, particularly with regard to the

nature of the barrier mechanisms and the enhancement of penetration of organic solutes. In summary the following points appear to be justified:

(1) The major rate-limiting barrier to penetration is the cuticle waxes which are located on the outer side of the cuticular membrane.
(2) Solute penetration appears to involve at least three phases, including sorption, transcuticular transport and desorption into the apoplast.
(3) Sorption into the waxes is biphasic, a rapid initial phase being followed by a slower, prolonged phase.
(4) Transport across the waxes appears to occur by diffusion via an amorphous wax matrix; route tortuosity is increased by the presence of crystalline wax.
(5) The rate of cuticular penetration is influenced directly or indirectly by a number of factors, these being chemical, plant or environmental in origin.
(6) Experimental models have been developed involving isolated cuticles, isolated waxes or detached leaves (eg conifer leaves) enabling calculation of solute flow (P) across the cuticular membrane using partition (K), diffusion (D) coefficients and rate constants of desorption (k^*); path length and concentration gradient are important parameters.
(7) Adjuvants may be used to enhance the rate and efficiency of cuticle penetration apart from their effects on interfacial tension; surfactants may be highly effective as activators and enhancers.
(8) Adjuvants known as accelerators (eg tributylphosphate or ethoxylated decanols) appear to change wax viscosity and their action is independent of solute polarity.
(9) The transport of solutes with moderate to high log P values (eg diflufenican, log P 4.6) may be enhanced by the action of accelerators in plasticising or elasticising the lipid components, while penetration of more polar compounds may be facilitated by hydration of the cuticle.
(10) Currently, optimum combinations of solute and adjuvant have to be found empirically; for predictive modelling more information is required on the rates of penetration of accelerators, and the solubilities of test solutes in cuticular waxes and adjuvants. Generation of these data should enable prediction of good solute/accelerator combinations and reduce the need for empirical testing.
(11) In-vivo studies have enabled examination of the uptake (and distribution) of ^{14}C-labelled organic solutes. While the role of the cuticle is less clearly focused, this approach enables identification of rate-limiting factors influencing target site deliveries. Using in-vivo studies a predictive model developed by Holloway and Stock[76] has enabled some optimisation of surfactant choice according to the solute log P.
(12) In-vitro and in-vivo approaches have been invaluable in developing our understanding of the plant cuticle; perhaps future studies should include more effort to bridge the apparent gap between the foliar uptake of AI by whole plants and in-vitro cuticle model systems.

REFERENCES

1 Kirwood RC, Pathways and mechanisms of uptake of foliage-applied herbicides with particular reference to the role of surfactants, in *Target Sites for Herbicide Action*, Ed by Kirkwood RC, Plenum Press, London. pp 219–243 (1991).
2 Holloway PJ, Structure and histochemistry of plant cuticular membranes: An overview, in *The Plant Cuticle*, Ed by Cutler DF, Alvin KL and Price CE, Linn Soc. Symp. Series No 10, Academic Press, London. pp 1–32 (1982).
3 Baker EA, Chemistry and morphology of plant epicuticular waxes, in *The Plant Cuticle*, Ed by Cutler DF, Alvin KL and Price CE, Linn Soc Symp Series No 10, Academic Press, London. pp 139–165 (1982).
4 Price CE, A review of the factors influencing the penetration of pesticides through plant leaves, in *The Plant Cuticle*, Ed by Cutler DF, Alvin KL and Price CE, Linn Soc. Symp. Series No 10, Academic Press, London. pp 237–252 (1982).
5 Kirkwood RC, Some criteria determining penetration and translocation of foliage-applied herbicides, in *Herbicides and Fungicides, Factors Affecting Their Activity*, Ed by McFarlane NR, Special Publication No 29, The Chemical Society, London. pp 67–80 (1977).
6 Holloway PJ, Structure and chemistry of plant cuticles. *Pestic Sci* **37**:203–232 (1993).
7 Jeffree CE, Structure and ontogeny of plant cuticles, in *Plant Cuticle: an integrated functional approach*, Environmental Plant Biology Series, Ed by Kerstiens G, BIOS Scientific Publishers Ltd, Oxford. pp. 33–82 (1996).
8 Canet D, Rohr R, Chamel A and Guillain F, Atomic force microscopy study of isolated ivy leaf cuticles observed directly and after embedding in Epon.® *New Phytol* **134**:571–577 (1996).
9 Riederer M and Markstädter C, Cuticular waxes: a critical assessment of current knowledge, in *Plant Cuticle: an integrated functional approach*, Environmental Plant Biology Series, Ed by Kerstiens G, BIOS Scientific Publishers Ltd, Oxford. pp. 189–200 (1996).
10 Kolattukudy PE and Espelie KE, Chemistry, biochemistry and function of suberin and associated waxes. In *Natural Products of Woody Plants*, Ed by Rowe JW, Springer-Verlag, Berlin. pp 304–367 (1989).
11 Bianchi G, Plant waxes, in *Waxes: Chemistry, Molecular Biology and Functions*, Ed by Hamilton RJ, The Oily Press, Dundee. pp 175–222 (1995).
12 Kolattukudy PE, Biosynthetic pathways of cutin and their waxes, and their sensitivity to environmental stresses, in *Plant Cuticle: an integrated functional approach*, Environmental Plant Biology Series, Ed by Kerstiens G, BIOS Scientific Publishers Ltd, Oxford. pp 83–108 (1996).
13 Riederer M and Schneider G, Comparative study of the composition of waxes extracted from isolated leaf cuticles and from whole leaves of citrus – evidence for selective extraction. *Physiol Plant* **77**:373–384 (1989).

14 Shepherd T, Robertson GW, Griffiths DW, Birch ANE and Duncan G, Effects of environment on the composition of epicuticular wax from kale and swede. *Phytochemistry* **40**:407–417 (1995).
15 Markstädter C, *Doct Diss Universitat Kaiserslautern*, Germany (1994).
16 Cassagne C and Lessire R, Studies on alkanal biosynthesis in epidermis of *Allium porrum* L. leaves. IV. Wax movement into and out of the epidermal cells. *Plant Sci Letters* **5**:261–268 (1975).
17 Hall DM and Donaldson LA, Secretion from pores of surface wax on plant leaves. *Nature* (*London*) **194**:1196 (1962).
18 Sargent C, In-situ assembly of cuticular wax. *Planta* **120**:123–126 (1976).
19 Miller RH, The prevalence of pores and canals in leaf cuticular membranes. *Ann Bot* **55**:459–471 (1985).
20 Jeffree CE, Baker EA and Holloway PJ, Ultrastructure and recrystallisation of plant epicuticular waxes. *New Phytol* **75**:539–549 (1975).
21 Jeffree CE, Baker EA and Holloway PJ, Origins of the fine structure of plant epicuticular waxes, in *Microbiology of aerial plant surfaces*, Ed by Dickinson, T. F. Academic Press, London, 1976, pp. 119–158.
22 Chambers TC, Ritebie IM and Booth MA, Chemical models for plant wax morphogenesis. *New Phytol* **77**:43–49 (1976).
23 Jeffree CE, The cuticle, epicuticular wax and trichomes of plants, with reference to their structure, function and evolution, in *Insects and the plant surface*, Ed by Juniper BF and Southwood TRE, Arnold, London. pp 23–64 (1986).
24 Anton LA, Ewers FW, Hammerschmidt R and Klomparens KL, Mechanisms of deposition of epicuticular wax in leaves of broccoli, *Brassica oleracea* L. var. capitata L. *New Phytol* **126**:505–510 (1994).
25 Kern AJ, Jackson LL and Dyer WE, Fatty acid and wax biosynthesis in susceptible and triallate-resistant *Avena fatua* L. *Pestic Sci* **51**:21–26 (1997).
26 McWhorter CG, Epicuticular wax on johnsongrass (*Sorghum halepense*) leaves. *Weed Sci* **41**:475–842 (1993).
27 McWhorter CG, Paul RN and Ouzts JC, Bicellular trichomes of johnsongrass (*Sorghum halepense*) leaves: morphology, histochemistry, and function. *Weed Sci* **43**:201–208 (1995).
28 Chambers GV, Bulawa MC, McWhorter CG and Hanks JE, Use of surface relationships models to predict the spreading of nonaqueous droplets on johnsongrass. In *Pesticide Formulations and Applications Systems*, Ed by Chasin DG and Bode LE, ASTM STP 1112, Philadelphia, PA. pp 218–246 (1992).
29 Watanabe T and Yamaguchi I, Studies on wetting phenomena on plant leaf surfaces 3: A retention model for droplets on solid surfaces. *Pestic Sci* **34**:273–279 (1992).
30 Grayson BT, Webb JD, Pack SE and Edwards D, Development and assessment of a mathematical model to predict foliar spray deposition from a laboratory track sprayer. *Pestic Sci* **33**:281–304 (1991).
31 Grayson BT, Pack SE, Edwards D and Webb JD, Assessment of a mathematical model to predict foliar spray deposition under laboratory track spraying conditions. II Examination with further plant species and diluted formulations. *Pestic. Sci* **37**:133–140 (1993).
32 Schreiber L and Schönherr J, Uptake of organic chemicals in conifer needles: Surface adsorption and permeability of cuticles. *Environ Sci Technol* **26**:153–159 (1992).
33 Bukovac MJ and Petracek PD, Characterizing pesticide and surfactant penetration with isolated plant cuticles. *Pestic Sci* **37**:179–194 (1993).
34 Kerler F, Riederer M and Schönherr J, Non-electrolyte permeability of plant cuticles: A critical evaluation of experimental methods. *Physiol Plant* **62**:599–602 (1984).
35 Schönherr J and Riederer M, Foliar penetration and accumulation of organic chemicals in plant cuticles. *Rev Environ Contam Toxicol* **108**:1–70 (1989).
36 Chaumat E, Chamel E, Taillandier G and Tissut M, Quantitative relationships between structure and penetration of phenylurea herbicides through isolated plant cuticles. *Chemosphere* **24**:189–200 (1992).
37 Chaumat E and Chamel A, Sorption and permeation to phenylurea herbicides of isolated cuticles of fruit and leaves. Effects of cuticular characteristics and climatic parameters. *Chemosphere* **22**:85–97 (1991).
38 Schönherr J and Riederer M, Desorption of chemicals from plant cuticles: Evidence for asymmetry. *Arch Environ Contam Toxicol* **17**:13–19 (1988).
39 Schreiber L and Schönherr J, Uptake of two chlorinated chemicals in conifer needles: Reversibility and compartmental analysis. *New Phytol* **123**:547–554 (1993).
40 Schreiber L and Schönherr J, Mobilities of organic compounds in reconstituted cuticular wax of barley leaves: determination of diffusion coefficients. *Pestic Sci* **38**:353–361 (1993).
41 Schreiber L, A mechanistic approach towards surfactant/wax interactions: effects of octaethyleneglycol monodecylether on sorption and diffusion of organic chemicals in reconstituted cuticular wax of barley leaves. *Pestic Sci* **45**:1–11 (1995).
42 Schreiber L and Riederer M, Determination of diffusion coefficients of octadecanoic acid in isolated cuticular waxes and their relationship to cuticular water permeabilities. *Plant Cell Environ* **19**:1075–1082 (1996).
43 Baur P and Schönherr J, Determination of mobilities of organic compounds in plant cuticles and correlation with molar volumes. *Pestic Sci* **35**:1–11 (1992).
44 Reynhardt EC and Riederer, M, Structure and molecular dynamics of the cuticular wax from leaves of *Citrus aurantiicum* L. *J Phys D: Appl Phys*. **24**:478–486 (1991).
45 Reynhardt EC and Riederer M, Structure and molecular dynamics of plant waxes. 2. Cuticular waxes from leaves of *Fagus sylvatica* L. and *Hordeum vulgare* L. *Eur Biophy J* **23**:57–60 (1994).
46 Riederer M and Schreiber L, Waxes: The transport barriers of plant cuticles, in *Waxes: Chemistry, Molecular Biology and Functions*, Ed by Hamilton RJ, The Oily Press, Dundee. pp 131–156 (1995).
47 Schreiber L, Kirsch T and Riederer M, Transport properties of cuticular waxes of *Fagus sylvatica* L. and *Picea abies* (L.) Karst: Estimation of size selectivity and tortuosity from diffusion coefficients of aliphatic molecules. *Planta* **198**:104–109 (1996).
48 Schreiber L, Kirsch T and Riederer M, Diffusion through cuticles: principles and models. In *Plant Cuticle: an integrated functional approach*, Ed by Kerstiens G, BIOS Scientific Publishers Oxford. pp 109–119 (1996).
49 Schönherr L and Baur P, Modelling penetration of plant cuticles by crop protection agents and effects of adjuvants on their rates of penetration. *Pestic Sci* **42**:185–208 (1994).
50 Baur P, Marzouk H and Schönherr L, Mobilities of organic compounds in plant cuticles as affected by structure and molar volumes of chemicals and plant species. *Planta* **199**:404–412 (1996).
51 Foy CL, Progress and development in adjuvant use since 1989 in the USA. *Pestic Sci* **38**:65–76 (1993).
52 Stock D and Holloway PJ, Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. *Pestic Sci* **38**:165–178 (1993).
53 Schönherr J, Effects of monodisperse alcohol ethoxylates on mobility of 2,4-D in isolated plant cuticles *Pestic Sci* **38**:155–164 (1993).
54 Schönherr J and Baur P, Effects of temperature, surfactants and other adjuvants on rates of uptake of organic compounds. In *Plant Cuticle: an integrated functional approach*, Ed by Kerstiens G, BIOS Scientific Publishers, Oxford. pp 135–155 (1996).
55 Baur P and Schönherr J, Temperature dependence of the diffusion of organic compounds across plant cuticles. *Chemosphere* **30**:1331–1340 (1995).

56 Schönherr J and Baur P, Cuticular permeability studies: A model for estimating leaching of plant metabolites to leaf surfaces, in *Aerial Plant Surface Microbiology*, Ed by Morris CE, Nicot PC and Nguyen-The C, Plenum Press, New York. pp 1–23 (1996).
57 Baur P, Buchholz A and Schönherr J, Diffusion in plant cuticles as affected by temperature and size of organic solutes: similarity and diversity among species. *Plant Cell Environ* **20:**982–994 (1997).
58 Baur P, Grayson BT and Schönherr J, Concentration-dependent mobility of chlorfenvinophos in isolated plant cuticles. *Pestic Sci* **47:**171–180 (1996).
59 Schönherr J and Baur P, Effect of tetraethyleneglycol monooctylether (C_8E_4) on mobilities of selected pesticides in *Citrus* leaf cuticles. *Z Pflanzenkrank Pflanzenschutz* **104:**246–253 (1997).
60 Baur P, Log-normal distribution of water permeability and organic solute mobility in plant cuticles. *Plant Cell Environ* **20:**167–177 (1997).
61 Gauvrit C and Cabanne F, Oils for weed control: uses and mode of action. *Pestic Sci* **37:**147–153 (1993).
62 Gauvrit C, Mercier L and Parmentier J, Influence of ester derivatives of oleic sunflower seed oil on the foliar penetration of herbicides. *Med Fac Landbouww Univ Gent* **60:**183–189 (1995).
63 Serre I, Cabanne F and Gauvrit C, Behaviour of alkyl oleates on leaf surfaces in relation to their influence on herbicide penetration. *Proc Brighton Crop Prot Conf – Pests and Diseases*, 807–812 (1996).
64 Langlois P, Gauvrit C, Monloungui Z and Darchy F, Fatty acid derivatives as co-formulants for herbicides. *Proc Brighton Crop Prot Conf – Weeds*, **1:**391–398 (1997).
65 Santier S and Chamel A, Penetration of triolein and methyl oleate through isolated plant cuticles and their effect on penetration of [^{14}C]quizalfop-ethyl and [^{14}C]fenoxaprop-ethyl. *Weed Res* **36:**167–174 (1996).
66 Coret J and Chamel A, Influence of some nonionic surfactants on water sorption by isolated tomato fruit in relation to cuticular penetration of glyphosate. *Pestic Sci* **38:**27–32 (1993).
67 Coret J, Gambonnet B, Brabet F and Chamel A, Diffusion of three ethoxylated octylphenols across isolated plant cuticles. *Pestic Sci* **38:**201–209 (1993).
68 Coret J and Chamel A, Effect of some ethoxylated alkylphenols and ethoxylated alcohols on the transfer of [^{14}C]chlorotoluron across isolated plant cuticles. *Weed Res* **34:**445–451 (1994).
69 Manthey FA and Nalewaja JD, Relative wax solubility and phytotoxicity of oil in green foxtail. In *Adjuvants for Agrichemicals* Ed by Foy CL, CRC Press, Boca Raton. pp 463–471 (1992).
70 Gauvrit C, *Interactions between adjuvants, agrochemicals and target organisms*, Ernest Schering Research Foundation Workshop 12, Ed by Holloway PJ, Rees R and Stock D, Springer Verlag, Berlin. pp 171–191 (1994).
71 Kirkwood RC, Use and mode of action of adjuvants for herbicides: A review of some current work. *Pestic Sci* **38:**93–102 (1993).
72 Stock D, Holloway PJ, Grayson BT and Whitehouse P, Development of a predictive uptake model to rationalise selection of polyoxyethylene surfactant adjuvants for foliage-applied agrochemicals. *Pestic Sci* **37:**233–245 (1993).
73 Holloway PJ, Wong WW-C, Partridge HJ, Seaman D and Perry RB, Effect of some nonionic polyoxyethylene surfactants on uptake of ethirimol and diclobutrazol from suspension formulations applied to wheat leaves. *Pestic Sci* **34:**109–118 (1992).
74 Gaskin RE and Holloway PJ, Some physiochemical factors influencing foliar uptake enhancement of glyphosate-mono(isopropylammonium) by polyoxyethylene surfactants. *Pestic Sci* **34:**195–206 (1992).
75 Stock D, Edgerton BM, Gaskin RE and Holloway PJ, Surfactant-enhanced foliar uptake of some organic compounds: interactions with two model polyoxyethylene aliphatic alcohols. *Pestic Sci* **34:**233–242 (1992).
76 Holloway PJ and Stock D, Factors affecting the activation of foliar uptake of agrochemicals by surfactants, in *Industrial Applications of Surfactants* II, Ed by Karsa DR, Special Publication No 77, Royal Society of Chemistry, Cambridge. pp 303–337 (1990).
77 Sharma SD, Kirkwood RC and Whateley T, Effect of nonionic nonylphenol surfactants on surface physiological properties, uptake and distribution of asulam and diflufenican. *Weed Res* **36:**227–239 (1996).
78 Chamel AR, Gambonnet B and Coret J, Effects of two ethoxylated nonylphenols on sorption and penetration of [^{14}C]isoproturon through isolated plant cuticles. *Plant Physiol Biochem* **30:**713–721 (1992).
79 Silcox D and Holloway PJ, Foliar absorption of some nonionic surfactants from aqueous solutions in the absence and presence of pesticidal active ingredients, in *Adjuvants and Agrochemicals*, Ed by Chow PNP, Grant CA, Hinshalwood AM and Simundsson E, Vol 2, CRC Press Boca Raton Fl. pp 115–128 (1989).
80 Knoche M and Bukovac MJ, Studies on octylphenoxy surfactants: XI. Effect of NAA diffusion through the isolated tomato fruit cuticular membrane. *Pestic Sci* **38:**211–217 (1993).
81 Riederer M and Schönherr J, Effects of surfactants on water permeability of isolated plant cuticles and composition of cuticular waxes. *Pestic Sci* **29:**85–94 (1990).
82 Baur P, Marzouk H, Schönherr J and Grayson BT, Partition coefficients of active ingredients between plant cuticle and adjuvants in relation to rates of foliar uptake. *J Agric Food Chem* **45:**3659–3665 (1997).